APPENDIX C - ADDITIONAL TRAINING AIDS

"Rapid and Efficient Resolution of Parentage by Amplification of Short Tandem Repeats," R. L. Alford, H. A. Hammond, I. Coto, and C. T. Caskey, American Journal of Human Genetics, Vol. 55: 190-195, 1994.

STR loci occur throughout the genome at an estimated frequency of one STR every 300-500 kb.

"Progress in a Genome Scan for Linkage in Schizophrenia in a Large Swedish Kindred," C. L. Barr, American Journal of Medical Genetics (Neuropsychiatric Genetics), Vol. 54: 51-58, 1994.

STRs are used in diagnostic, clinical, and genetic mapping of such medical disorders as Schizophrenia, as well as for linkage and linkage disequilibrium mapping.

"Instability of Short Tandem Repeats (Microsatellites) in Human Cancers," R. Wooster and A. M. Cleton-Jansen, Nature Genetics, Vol 6: 152-156, 1994.

The allele sizes of polymorphic microsatellite repeats in DNA from human cancers were compared to normal DNA from the same patients. In 16 out of 196 paired samples evidence of an extra allele of a different size in the tumor was found which was not present in the normal DNA.

Based on the study that was conducted the authors found that there is instability of microsatellite repeats in several human cancers (Myotonic Dystrophy, X-linked spinal and bulbar muscular atrophy [Kennedy's syndrome], Huntington's disease, breast cancer, soft tissue sarcoma, brain cancer, and ovarian cancer).

One of the loci that was examined included the tetranucleotide vWA (found within an intron of the gene encoding von Willebrand's factor).

Approximately 10% of breast cancers, sarcomas and ovarian cancers exhibited additional alleles.

Because PCR amplification can sometimes generate spurious bands, each experiment in which an extra allele was detected was repeated at least three times. The results were consistent in all the experiments.

"Slippage Synthesis of Simple Sequence DNA," Christian Schlotterer and Diehthard Tautz, Nucleic Acids Research, Vol. 20: 211-215, 1992.

Slippage synthesis occurs in vivo on a fixed template where only one strand is free to move, a situation which resembles chromosome replication. It seems therefore likely that slippage during replication is the cause of the observed length polymorphisms between individuals in a population.

As the size of the repeat unit increases from a di- or tri-, to a tetranucleotide repeat, the growth of the repeat products is slower, which suggests that the slippage rate is slower. Different possible combinations of nucleotide repeat motifs have been examined and it has been demonstrated that they all grow at different rates. AT repeats grow faster than GC repeats, indicating that slippage is potentially dependent on the AT-content of the sequences involved.

"The Evolutionary Dynamics of Repetitive DNA in Eukaryotes," Brian Charlesworth, Paul Sniegowski, and Wolfgang Stephen, Nature, Vol. 371, September 15, 1994.

The behavior of repetitive sequences can result in mutations that cause genetic diseases which confer significant fitness losses on the organism.

In vitro studies suggest that strand slippage during DNA replication is the major cause of the observed length polymorphism of microsatellites within populations.

Definitions:

Microsatellite sequences: arrays of short (2-5 bp) nucleotide repeats found in vertebrate, insect and plant genomes. At least 30,000 microsatellite loci are present in the human genome. Copy numbers are characteristically variable within a population.

Minisatellite sequences: arrays of longer (~ 15 bp) repeats, generally involving mean array lengths of 0.5-30 kb. They are found in the genome of vertebrates, fungi, and plants, and are highly variable in size.

"Substrate Nucleotide-Determined Non-Template Additions for Adenine by *Taq* DNA Polymerase: Implications of PCR-Based Genotyping and Cloning," V. L. Magnuson, D. S. Ally, S. J. Nylund, Z. E. Rayman, J. I. Knapp, A. L. Lowe, S. Ghosh, and F. S. Collins, BioTechniques 21: 700-709, 1996.

Certain terminal nucleotides can either inhibit or enhance adenine addition by *Taq* and the PCR primer design can be used to modulate this activity.

Since the 5' end of the forward primer carries the fluorescent label, it is only this strand that is detected by the FMBIO.

For some PCR products, extended time at 4^0C or room temperature is enough to allow the PCR to proceed further towards the allele + A (5%-25% after 2 days at 4^0C).

As a general rule, it was demonstrated that when the 5' end nucleotide of the reverse primer was replaced with a "T", a 3' "A" terminal nucleotide would occur on the forward strand. The presence of an adenine on the 3' end of the PCR product is inhibitory to adenine addition by *Taq* DNA polymerase. The removal of "A" as terminal substrate nucleotide markedly enhances the PCR product to proceed to $\geq$ 70% allele + A.

"Sequence Analysis and Characterization of Stutter Products at the Tetranucleotide Repeat Locus vWA," P. Sean Walsh, Nicola J. Fildes, and Rebecca Reynolds, Nucleic Acids Research, Vol. 24: 2807-2812, 1996.

Definition:

Stutter: The PCR amplification of tetranucleotide short tandem repeat (STR) loci produces a minor product band 4 base pairs shorter than the corresponding main allele band. This is also referred to in the literature as shadow bands, DNA polymerase slippage product, or n-4 bands.

PCR amplification results from tetranucleotide repeat loci are easier to interpret than dinucleotide repeats because only a single band versus possible multiple bands for dinucleotides is observed in a position four bases shorter than each allele band, and the intensity of the stutter band is generally <10% of the main band.

The proportion of stutter product relative to the main allele increases as the number of uninterrupted core repeat units increases.

The most common repeat motif in the variable expansion region of a STR locus is referred to as the "core" repeat sequence.

The proportion of stutter peak can be overestimated for alleles that are 8 base pairs longer than the other alleles in the same sample (heterozygote). The overestimation is due to the fact that the stutter peak resides on the shoulder of the peak for the shorter allele. It may be possible to minimize this effect by running longer gels or implementing other gel condition modifications that increase peak sharpness or resolution.

Taq Polymerase has no 3' → 5' exonuclease (proofreading) activity, but does have a 5' → 3' exonuclease activity.

The mechanism known as slipped strand mispairing has been proposed to explain the stutter bands that result from amplification. According to this proposal, the template strand and extending strand can breath apart during synthesis through the repeat region, perhaps when/if the DNA polymerase has fallen off during PCR. A single repeat unit can then loop out in the template strand before the two strands re-anneal. The result is that the newly extended strand will have one fewer repeat unit than the template strand when synthesis is complete.

It is also conceivable in slipped strand mispairing that the extending strand could loop out, thus resulting in a newly extended strand having an additional repeat unit relative to the template strand. Even though this is possible, the most prevalent stutter bands are shorter than the main allele.

A speculative explanation for the relative lack of longer stutter bands is that the DNA polymerase may associate with the stretch of DNA at the 3' end of the extending strand when the strands are unpaired. This association may then somehow inhibit loop out formation in the extending strand.

It is possible that the template strand has more of an opportunity and/or tendency to loop out when long stretches of core repeats are present. Also the strand alignment that exists when loop out does occur in an interrupted core repeat stretch is less likely to position the 3' end of the extending strand across from a complementary base. The polymerase then would not complete synthesis of what would otherwise become the shortened, stutter strand.

However in the case of vWA and most other tetranucleotide repeat loci, stutter bands longer than the main allele have not been observed.

When choosing loci to incorporate into a multiplex to reduce the overall amount of stutter, it is recommended to choose loci whose alleles contain overall fewer repeat units and a core repeat that is interrupted by a four base pair sequence substitution.

"*GenePrint*™ PowerPlex™ 1.1 System" and "*GenePrint*™ Fluorescent STR Systems" Manuals, Promega, 9/97 and 8/97.

Due to the unusual number of chromosomes which can be represented more than twice per cell in the K562 cell line strain, the alleles within a locus can be different intensities. It is hypothesized that in this strain, the allele 5 version of chromosome 6 is present twice, while the allele 4 version of the chromosome 6 is present only once. These variations seen between alleles within a locus are not a consequence of primer imbalance during amplification.

The fluorescent ladder CXR (60-400 bases) contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, and 400 bases in length.

"Defining Microsatellite Alleles by Genotyping Global Indigenous Human Populations and Non-Human Primates," Li Jinj, Peter Underhill, Martin Buoncristiani, and James M. Robertson, Journal of Forensic Sciences, Vol. 42: 496-499, 1997.

Chimpanzee DNA cannot be amplified at the D7S820 locus. This is either due to a nucleotide substitution(s) at priming regions or an absence of the locus in the Chimp genome.

D5S818 alleles in Chimpanzees differ by increments of two base pairs instead of four, suggesting that there may be a dinucleotide repeat associated with the locus.

"Automated Fluorescent Detection of STR Multiplexes - Development of the *GenePrint™* PowerPlex™ and FFFL Multiplexes for Forensic and Paternity Applications," James W. Schumm, Ann M. Lins, Katherine A. Micka, Cynthia J. Sprecher, Dawn R. Rabbach, and Jeffrey W. Bacher, in Proceedings from the First European Symposium on Human Identification, pp. 90-104, 1996.

Repeat slippage, sometimes called "n-4" or "shadow" banding, occurs as loss of a repeat unit during DNA synthesis through regions of repeated sequences. The amount of this artifact observed is dependent primarily on the locus and the DNA strand.

Terminal nucleotide addition: the polymerase comes to the end of the amplified fragment, then adds a nucleotide, generally adenine, as an extra base. This activity often does not occur with 100% efficiency and varies with different primer sequences. Thus, an artifact band one base shorter than expected is sometimes seen. Redefinition of the primer sequence and/or adding a final extension step in the amplification protocol (60^0C for 30 minutes) can lead to conditions of essentially full terminal nucleotide addition.

Overall there appears to be a correlation between a high degree of polymorphism, a tendency for microvariants, and an increased mutation rate. For this reason, we work with loci with moderately high polymorphism..

WHAT IS MEANT BY A NON-NUCLEOTIDE ADDITION?

A terminal nucleotide addition occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the end of amplified DNA fragments in a template-independent manner. Thus, an artifact band, one base shorter than expected (i.e. missing the terminal addition), is sometimes seen. This activity often does not occur with 100% efficiency and varies with different primer sequences.

IF YOU WERE DESIGNING PRIMERS FOR PCR AMPLIFICATION, HOW WOULD YOU DESIGN THE REVERSE PRIMER TO REDUCE THE AMOUNT OF NON-TEMPLATE NUCLEOTIDE ADDITIONS? WHY?

As a general rule, it was demonstrated that when the 5' end nucleotide of the reverse primer was replaced with a "T", the result would be a 3' "A" terminal nucleotide on the forward strand. The presence of an adenine on the 3' end of the PCR product is inhibitory to adenine addition by *Taq* DNA polymerase. The removal of "A" as terminal substrate nucleotide markedly enhances the PCR product to proceed to $\geq$ 70% allele + A.

WHAT PORTION OF THE AMPLIFICATION CYCLE IS DESIGNED TO REDUCE NON-TEMPLATE NUCLEOTIDE ADDITIONS?

Addition of a final extension step at 60^0C for 30 minutes to the amplification protocol can lead to conditions of essentially full terminal nucleotide addition.

WHAT IS MEANT BY STUTTER?

The PCR amplification of tetranucleotide short tandem repeat (STR) loci produces a minor product band 4 base pairs shorter than the corresponding main allele band. This is also referred to in the literature as shadow bands or DNA polymerase slippage product.

WHAT CAUSES STUTTER?

The mechanism known as slipped strand mispairing has been proposed to explain the stutter bands that result from amplification. According to this proposal, the template strand and extending strand can breath apart during synthesis through the repeat region, perhaps when/if the DNA polymerase has fallen off during PCR. A single repeat unit can then loop out in the template strand before the two strands re-anneal. The result is that when synthesis is complete, the newly extended strand will have one fewer repeat unit than the template strand.

It is also conceivable in slipped strand mispairing that the extending strand could loop out, thus resulting in a newly extended strand having an additional repeat unit relative to the template strand. Even though this is possible, the most prevalent stutter bands are shorter than the main allele.

WHY IS IT MORE COMMON FOR THE TEMPLATE STRAND TO LOOP OUT VERSUS THE EXTENDING STRAND?

A speculative explanation for the relative lack of longer stutter bands is that the DNA polymerase may associate with the stretch of DNA at the 3' end of the extending strand when the strands are unpaired. This association may then somehow inhibit loop out formation in the extending strand.

It is possible that the template strand has more of an opportunity and/or tendency to loop out when long stretches of core repeats are present. Also the strand alignment that exists when loop out does occur in an interrupted core repeat stretch is less likely to position the 3' end of the extending strand across from a complementary base. The polymerase then would not complete synthesis of what would otherwise become the shortened, stutter strand.

TRUE OR FALSE:
***TAQ* POLYMERASE HAS A 3' → 5' EXONUCLEASE (PROOFREADING) ACTIVITY.** False

TRUE OR FALSE:
THE MOST COMMON REPEAT MOTIF IN THE VARIABLE EXPANSION REGION OF A STR LOCUS IS REFERRED TO AS THE "CORE" REPEAT SEQUENCE. True

TRUE OR FALSE:
THE 5' END OF THE REVERSE PRIMER CARRIES THE FLUORESCENT LABEL, WHICH IS DETECTED BY THE FMBIO. False, the 5' end of the forward primer is labeled.

WHEN CHOOSING LOCI TO INCORPORATE INTO A MULTIPLEX TO REDUCE THE OVERALL AMOUNT OF STUTTER, IT IS RECOMMENDED TO CHOOSE:

A. Loci whose alleles contain overall fewer repeat units
B. Loci that contain dinucleotide repeats
C. Loci that contain a core repeat that is uninterrupted by a four base pair sequence substitution
D. A and C
E. All of the above

D. A and C

DEFINE MICROVARIANT AND GIVE AN EXAMPLE OF A MICROVARIANT.

Alleles differing from one another by lengths other than the repeat length. Examples: TH01 9.3 and 8.3

WHAT ARE SOME OF THE REASONS FOR "FUZZY" BANDS THROUGHOUT THE GEL?

1. Poor quality polyacrylamide gel
2. Electrophoresis temperature is too high

EXPLAIN THE NOMENCLATURE THAT IS USED TO CALL AN ALLELE AT A PARTICULAR LOCUS, FOR EXAMPLE, FGA 22 VERSUS FGA 22.2.

Alleles are assigned based upon their repeat lengths. The FGA 22.2 allele is a microvariant with 22 complete repeats, with an incomplete repeat of an additional 2 bases.

AS A GENERAL RULE, WE WORK WITH STR LOCI WITH MODERATELY HIGH POLYMORPHISM. WHY DO WE NOT WORK WITH HIGHLY POLYMORPHIC LOCI?

There appears to be a correlation between a high degree of polymorphism and a tendency for microvariants and increased mutation rates.

I GENERAL MOLECULAR BIOLOGY QUESTIONS

1. Approximately how many base pairs are found in a single human diploid cell?
2. DNA is found both in the ? and the ? of a typical eukaryotic cell.
3. What is one difference between a prokaryote and eukaryote cell?
4. What is the name of the process by which a diploid parent cell gives rise to two diploid daughter cells?
5. A change in a wild type genetic sequence is commonly referred to as a ? .
6. Who is known as the "Father of Genetics"?
7. If an individual has a genetic profile consisting of the same alleles at a locus (e. g., CSF1PO 12, 12), he is said to be ? at this locus.
8. If an individual has a genetic profile consisting of different alleles at a locus (e. g., TH01 8, 10), he is said to be ? at this locus.
9. Replication of DNA is accomplished using an enzyme called a ? .
10. DNA replication involves the disruption of the double helix at a junction known as the ? .
11. With regard to DNA replication, synthesis occurs in what direction?
12. The enzyme responsible for unwinding the DNA in preparation for replication is called ? .
13. An enzyme which cleaves nucleotides from the end of a DNA chain is called a ? .
14. A triplet of nucleotides in a DNA sequence representing an amino acid is called a ? .
15. Proteins are composed of linked organic molecules called ? .
16. DNA is cloned by inserting sequence fragments into circular DNA vectors called ? .
17. What are the names of the four bases found in DNA?

Define the following terms:

18. Phenotype
19. Genotype
20. Haploid cell
21. Diploid cell
22. Gamete

23. Zygote

24. Allele

25. Locus

26. Chromosome

27. Genome

28. Base Pair

29. Tm

30. Which of the following would have a higher Tm and why?

    A. CTGTTTTTGCAATGCAATATTAC
    B. AGCCCCCATTTCGGGCGGCCCCG

31. What is the difference between a nucleotide and a nucleoside?

32. What is the basic difference between an intron and an exon?

33. What is the difference between mRNA sequences and their DNA counterpart sequences?

I ANSWERS TO GENERAL MOLECULAR BIOLOGY QUESTIONS

1. 6 billion
2. Nucleus and Mitochondria
3. Nucleus
4. Mitosis
5. Mutation
6. Gregory Mendel
7. Homozygote
8. Heterozygote
9. Polymerase
10. Replication fork
11. 5' → 3'
12. Helicase
13. Exonuclease
14. Codon
15. Amino Acids
16. Plasmids
17. Adenine, Thymine, Guanine, and Cytosine
18. Phenotype: The physical characteristic expressed as a result of the genotype
19. Genotype: The pair of alleles at a locus or set of loci
20. Haploid cell: A cell containing a complement of DNA for a given species (Example: one half of a diploid cell)
21. Diploid cell: A cell containing the full complement of DNA from a female and male
22. Gamete: Sex cell (Examples: sperm and eggs)
23. Zygote: Union of a sperm and egg to make a single diploid cell
24. Allele: Alternative form or version of a gene; a sequence difference could account for this form

25. Locus: A specific physical position on a chromosome at which a gene or gene pair reside

26. Chromosome: The package containing a combination of genetic material (DNA) and proteins.

27. Genome: The total genetic complement of an organism defined by one copy of the DNA found in each cell.

28. Base Pair: The bonding of an Adenine (A) to Thymine (T) and Cytosine (C) to Guanine (G)

29. Tm: Melting temperature (i. e., the temperature at which double stranded nucleic acids dissociate)

30. "B" since there are more G:C bonds

31. A nucleotide has one more phosphate group than a nucleoside.

32. Introns are non-transcribed DNA sequences where exons are transcribed into proteins.

33. mRNA contains no introns; it carries the code to make a protein.

II GENETICS PROBLEMS AND QUESTIONS

1. A gene for a particular genetic characteristic for hemoglobin production can be inherited in two forms: "Z" or "z". "Z" is dominant and when expressed, offspring will have normal hemoglobin levels. Recessive "z" always results in high hemoglobin levels. A second genetic characteristic for the trait "Progragy" is inherited as "X" where "X" is dominant and non-lethal. Recessive "x" homozygote is always lethal within the first decade of a child's life.

   A mother is known to have the genetic profile "ZZ" and "Xx" and the father has the profile "Zz" and "Xx".

   A. Prepare a Punnet square diagram to demonstrate the possible patterns of inheritance for these two traits considering the mother's and father's profiles described above.

   B. Based on the Punnet square data that was generated for the "Progragy" trait, what is the possible ratio of children who may die to children who will live within the first decade of life?

   C. What would it mean if the literature has reported that these two genetic traits are in linkage disequilibrium?

2. Name at least two types of genetic mutations that can occur in the DNA strand.

3. What is the difference between a "mutation" and a "polymorphic sequence"?

4. The complete DNA sequence of a new species of plant has recently been found in the rain forest.

   A. Using the conversion chart below, what would the protein sequence be if the following RNA sequence was obtained from the plant?

      5' AUG UUU GCU UUU CGG GGC CUA CUA AAA UAG 3'

B. What would the DNA sequence be for the above plant RNA sequence?

1st Position	2nd Position				3rd Position
	U	C	A	G	
U	PHE PHE LEU LEU	SER SER SER SER	TYR TYR STOP STOP	CYS CYS STOP TRP	U C A G
C	LEU LEU LEU LEU	PRO PRO PRO PRO	HIS HIS GLN GLN	ARG ARG ARG ARG	U C A G
A	ILE ILE ILE MET	THR THR THR THR	ASN ASN LYS LYS	SER SER ARG ARG	U C A G
G	VAL VAL VAL VAL	ALA ALA ALA ALA	ASP ASP GLU GLY	GLY GLY GLY GLY	U C A G

II ANSWERS TO GENETICS PROBLEMS AND QUESTIONS

1A.

		FATHER			
		ZX	Zx	zX	zx
MOTHER	ZX	ZZXX	ZZXx	ZzXX	ZzXx
	Zx	ZZXx	**ZZxx**	ZzXx	**Zzxx**

1B. 1:3, one child will die for every 3 that live

1C. Inheritance of the hemoglobin-related gene is linked to inheritance of the Progragy gene. The phenotype associated with either of these two traits is predictable.

2. Insertion: An addition of one or several nucleotides into the DNA strand.

Deletion: A chromosomal mutation characterized by the loss of a chromosome segment or a nucleotide

Transversion: A base pair substitution mutation resulting in the replacement of a purine by a pyrimidine, or vice versa.

Transition: A base pair substitution mutation resulting in the replacement of one purine by another purine, or a pyrimidine by another pyrimidine.

Duplication: A chromosomal mutation characterized by the presence of two copies of a chromosome segment in the haploid genome.

Inversion: A chromosomal mutation characterized by the reversal of a chromosome segment.

3. A mutation is any inheritable change in the DNA sequence that occurs during reproduction or cell division. A polymorphism is a genetic locus for which there are may alleles in existence based on the mutation.

4A. 5' MET PHE ALA PHE ARG GLY LEU LEU LYS STOP 3'

4B. 5' ATG TTT GCT TTT CGG GGC CTA CTA AAA TAG 3'
3' TAC AAA CGA AAA GCC CCG GAT GAT TTT ATC 5'

III POLYACRYLAMIDE GEL ELECTROPHORESIS QUESTIONS

1. List two characteristics of polyacrylamide that make it an ideal matrix to separate DNA molecules.

2. What is a "Polymer Web"?

3. What is the difference between the %T of a gel and the %C?

4. A protocol for preparing a polyacrylamide gel calls for 4.2g of acrylamide and 0.3g of bis acrylamide per 100 ml of buffer.

   A. What is the %T of this gel?

   B. What is the %C of this gel?

   C. Could this gel be used to separate PowerPlex alleles using a SA32 extended gel unit?

5. Would the following gel characteristics increase or decrease if too little TEMED was added to the PAG preparation?

   a. average polymer chain length
   b. gel turbidity
   c. gel elasticity
   d. polymerization time

6. Why is it important to gently mix the TEMED/AP with the Acrylamide:Bis and to degas the polyacrylamide solution before pouring the gel?

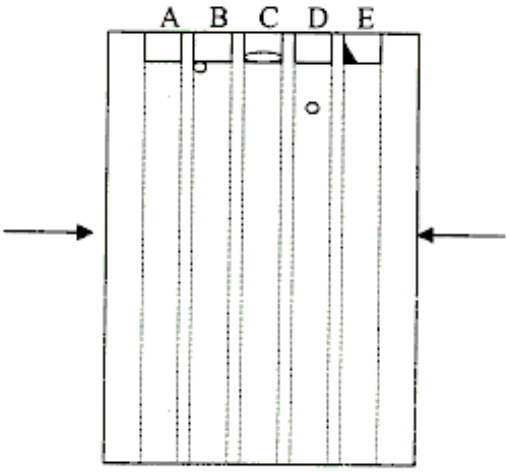
7. Why should preparation of the polyacrylamide and pouring the gel plates be conducted at approximately 23-25^{o}C?

8. List two polyacrylamide contaminants in the reagents that will affect the reproducibility of PAG.

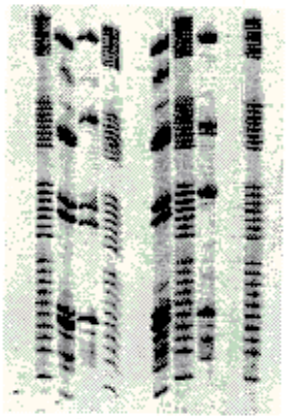
9. Assume the same amplified sample is being electrophoresed in each of the wells below. The sample consists of a single band 254 bp in length which will migrate to the arrows shown in the diagram. If the sample was allowed to migrate as far as the arrows (shown below), what would the sample band look like in wells "A" through "E" if you know the following?

Well	Problem
A	The sample was not adequately heated and cooled before loading.
B	There was a bubble in the well formation.
C	Urea was not flushed from the well.
D	A bubble was further down in the gel lane.
E	A piece of acrylamide was left in the well.

Draw a band adjacent to the arrows that would show the migration pattern of the DNA in each lane.



10. What might be a logical explanation for the following migration pattern?



11. When conducting precision analysis of your FMBIO instrument, you notice the following band pattern on your gel. You have run each sample with an internal lane standard. Can you use all of the lanes in your study? Explain your answer.

III ANSWERS TO POLYACRYLAMIDE GEL ELECTROPHORESIS QUESTIONS

1. List two characteristics of polyacrylamide that make it an ideal matrix to separate DNA molecules.

   a. Chemically and biologically inert
   b. Very few charge groups
   c. Excellent molecular sieving properties
   d. High tensile strength

2. What is a "Polymer Web"?

   TEMED > Formation of free radicals (FR) > Acrylamide monomers react with FR > Polymerization chain reaction > Bis cross linking > Polymer Web

3. What is the difference between the %T of a gel and the %C?

   %T measures the tensile strength which is based on chain lengths and %C measures the amount of bis cross linking

4. A protocol for preparing a polyacrylamide gel calls for 4.2g of acrylamide and 0.3g of bis acrylamide per 100 ml of buffer.

   A. What is the %T of this gel?

      4.2g + .0.3g / 100 x 100 = 4.5%T

   B. What is the %C of this gel?

      .0.3g / 4.2g + .0.3g x 100 = 6.7%C

   C. Could this gel be used to separate PowerPlex alleles using a SA32 extended gel unit?

      Yes

5. Would the following gel characteristics <u>increase</u> or <u>decrease</u> if too little TEMED was added to the PAG preparation?

   a. average polymer chain length — <u>Increase</u>
   b. gel turbidity — <u>Decrease</u>
   c. gel elasticity — <u>Increase</u>
   d. polymerization time — <u>Increase</u>

6. Why is it important to gently mix the TEMED/AP with the Acrylamide:Bis and to degas the polyacrylamide solution before pouring the gel?

   Oxygen may be introduced when mixing. Oxygen is a free radical trap; therefore, polymerization will be decreased in the presence of oxygen.

7. Why should preparation of the polyacrylamide and pouring the gel plates be conducted at approximately 23-25^{o}C?

Cold reagents trap oxygen. Very warm reagents initiate polymerization too quickly, which produces short monomer chain lengths.

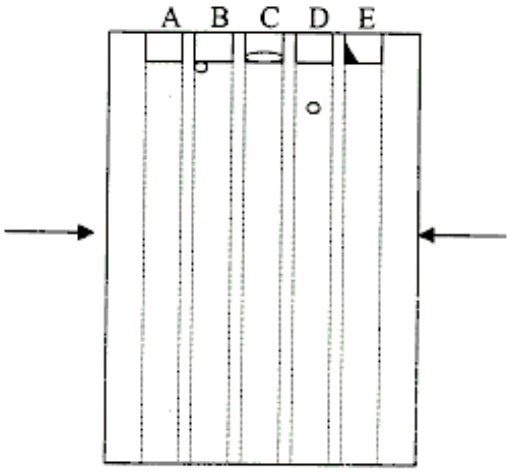
8. List two polyacrylamide contaminants in the reagents that will affect the reproducibility of PAG.

a. Acrylic acid
b. Oxygen
c. Ions
d. Linear polymers

9. Assume the same amplified sample is being electrophoresed in each of the wells below. The sample consists of a single band 254 bp in length which will migrate to the arrows shown in the diagram. If the sample was allowed to migrate as far as the arrows (shown below), what would the sample band look like in wells "A" through "E" if you know the following?

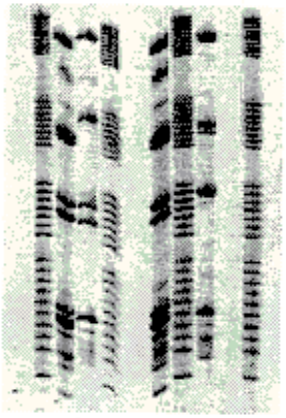
Well	Problem
A	The sample was not adequately heated and cooled before loading.
B	There was a bubble in the well formation.
C	Urea was not flushed from the well.
D	A bubble was further down in the gel lane.
E	A piece of acrylamide was left in the well.

Draw a band adjacent to the arrows that would show the migration pattern of the DNA in each lane.



10. What might be a logical explanation for the following migration pattern?

    The acrylamide was not completely polymerized in this area, maybe due to the presence of oxygen, bad bis, AP, or other impure reagents.



11. When conducting precision analysis of your FMBIO instrument, you notice the following band pattern on your gel. You have run each sample with an internal lane standard. Can you use all of the lanes in your study? Explain your answer.

Lanes 1, 2, and 6 could be used for the precision studies. Lanes 3, 4, and 5 should not be used, since the actual migration of the bands in these lanes cannot accurately be determined.

IV FMBIO QUESTIONS

Image Acquisition

1. After powering up, the FMBIO performs an autofocus routine just prior to the first read. In order to get the best results, the _____ nm filter should be in position # ____ when the system is first turned on.

2. If you were designing a two-color multiplex system for the FMBIO, which two of the following dyes would work best together? (For this question, assume that all dyes are excited with equal efficiency and emit at the same intensity.)

   (Circle only two)
   a. Dye A (ex 490, em 520)
   b. Dye B (ex 546, em 572)
   c. Dye C (ex 580, em 605)
   d. Dye D (ex 650, em 667)
   e. Dye E (ex 578, em 602)

3. The FMBIO allows the user to change the sensitivity of the photo multiplier tubes, depending on the dye being detected. Based on the 532 nm excitation source, which of the following dyes would most likely require a maximum (100%) detection sensitivity setting?

   (Circle only two)
   a. Dye A (ex 490, em 520)
   b. Dye B (ex 546, em 572)
   c. Dye C (ex 580, em 605)
   d. Dye D (ex 650, em 667)
   e. Dye E (ex 578, em 602)

4. Assuming a one-color detection system, choose the best filter for each dye. (You may use each answer more than once.)

   ___ Dye A (ex 490, em 520)
   ___ Dye B (ex 546, em 572)
   ___ Dye C (ex 580, em 605)
   ___ Dye D (ex 650, em 667)
   ___ Dye E (ex 578, em 602)

   a. 667 nm
   b. 585 nm
   c. 605 nm
   d. 625 nm
   e. 650 nm
   f. 505 nm

Image Analysis

Briefly define or explain the significance of the following:

5. Excitation wavelength

6. Emission wavelength

7. 65,536

Complete each of the following sentences by circling the correct word from each set of choices:

8. If horizontal resolution is changed from 300 dpi to 150 dpi:

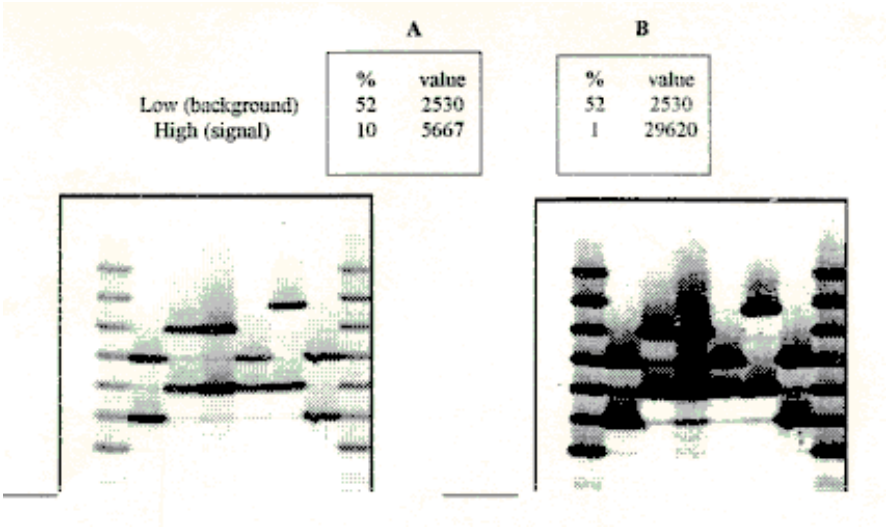
   the image becomes: sharper / more pixilated

   the image file size: decreases / increases

   the scan time: decreases / increases

9. For best results, when reading a typing gel with closely-spaced bands, horizontal / vertical resolution should be set at 75 / 150 / 300 dpi.

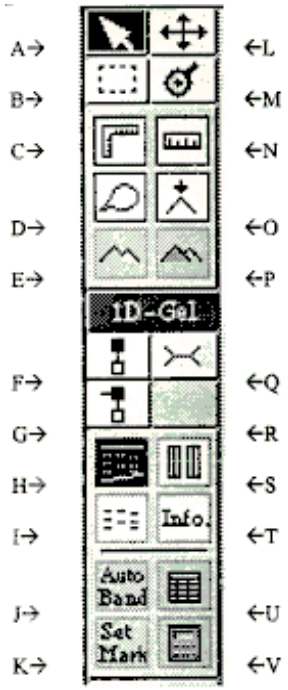
10. Match the image to the Gray Scale settings.



Explain what the "52%" Low (background) means.

Explain the difference between the 10% High (signal) and the 1% High (signal) as it relates to the gel scans above.

11. The following diagram represents commonly used icons on the FMBIO software. Match the function to the appropriate icon.



____a. Shows selected information next to bands on the gel file

____b. Magnifies the gel file picture

____c. Allows for manual addition of bands

____d. Defines bands within lanes automatically

____e. Comment icon, displays elements created in Fig Draw

____f. "Spectrum" tool, shows lane trace next to band image on the gel

____g. Single lane selection tool

____h. Peak finder tool, detects the densest area of a band

____i. Initiates calculation of band information

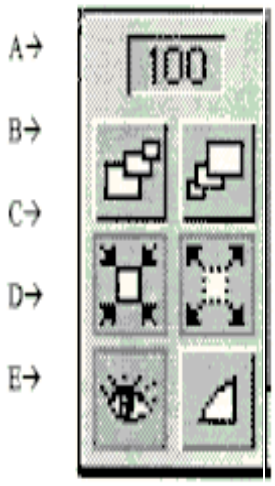
____j. Used to define many lanes at once

12. Referring to the diagram below:

____When clicked, which of the icons will sharpen a magnified image?

____Which of the icons fits a selected image to the size of the page?

____Which icon allows for alteration of the gray level on a gel file?



Circle the appropriate answer that will accurately complete the following sentence.

13. If using an in-lane size marker labeled with a dye different from that used to label sample bands, the Separate / Mix / Layer Marker Mode should be set.

14. When using allelic ladders for band sizing, Marker Mode can be set to:

a. Separate
b. Mix
c. Layer
d. a or b
e. a or c
f. a or b or c

15. Your colleague needs your help. During an analysis, very few of the bands that are visible to his eye were called using the AutoBand button. Which of the following might help him identify more bands?

    Mark true or false, and explain why or why not.

    _____ a. Choose AutoBanding Parameters and decrease the noise level.
    Explain:

    _____ b. Choose AutoBanding Parameters and increase the noise level.
    Explain:

    _____ c. Readjust any lane indicator lines that do not go through the middle of the lane.
    Explain:

    _____ d. Rescan the gel with the sensitivity increased.
    Explain:

    _____ e. Manually input bands with the >-< tool.
    Explain:

16. Although the greater majority of the allele calls in your colleague's analysis of his data in STaRCall™ has resulted in accurate allele calls, you notice there are several "not in range" messages for some bands. Which of the following could be causing this? (Check all that apply.)

    ____ The incorrect lookup table was used for the data being analyzed.

    ____ Internal size markers were incorrectly identified (325 bp band was identified as 350 bp, etc.).

    ____ In FMBIO Analysis, some bands in the allelic ladder lanes were incorrectly identified.

    ____ In the lookup table, +/- values were too small.

    ____ Overloaded lanes resulted in inaccurate migration distances.

    ____ Manually banding lanes without the peak finder tool resulted in bands not positioned correctly.

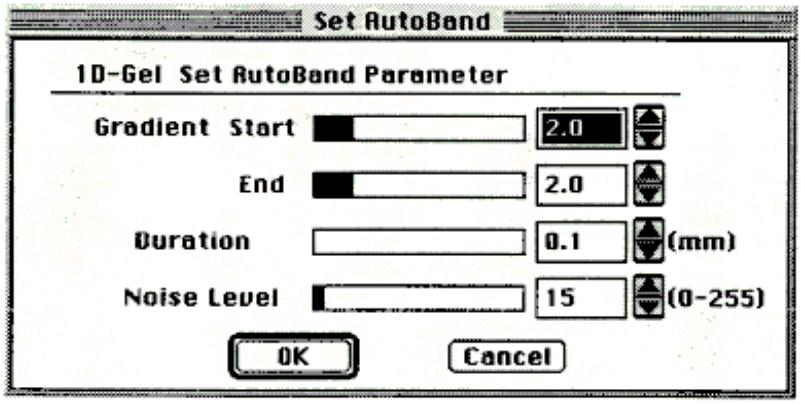
    ____ The gel was never color separated.

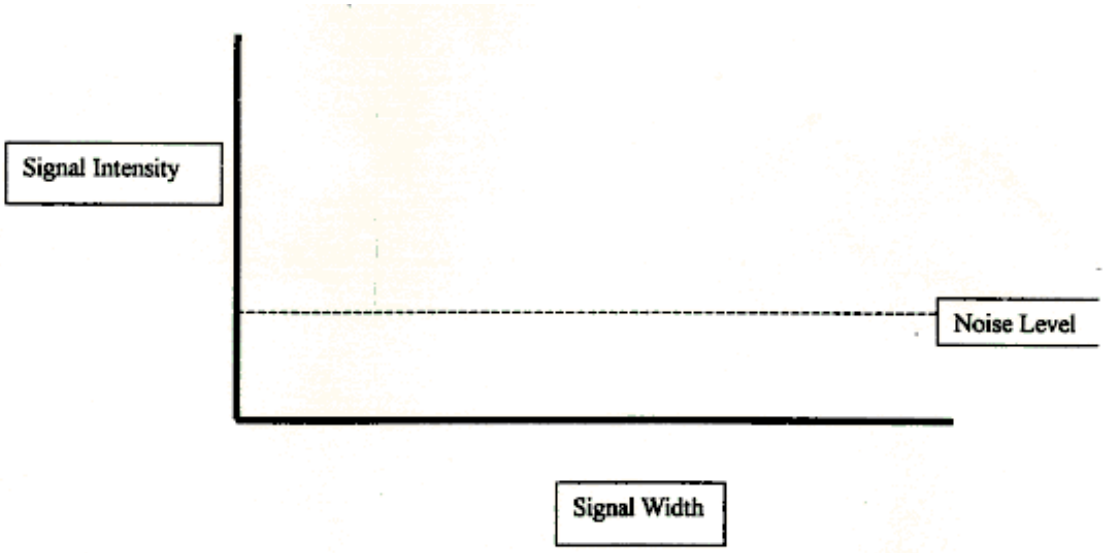
    ____ He has discovered new microvariants which are not present in the lookup table.

    ____ The wrong lookup table was used to evaluate a lane

    ____ Allelic ladders (instead of internal size markers) were used to size unknowns in a "smiling" or "frowning" gel.

17. Band recognition depends on four parameters: Gradient Start, Gradient End, Duration and Noise Level. FMBIO Analysis recognizes a signal peak as a band by using a spectrum curve. Based on the information given in the diagram below, fill in the spectrum curve below that represents the settings shown in the "Set AutoBand" window.





IV ANSWERS TO FMBIO QUESTIONS

Image Acquisition

1. After powering up, the FMBIO performs an autofocus routine just prior to the first read. In order to get the best results, the 598 nm filter should be in position # 1 when the system is first turned on.

2. If you were designing a two-color multiplex system for the FMBIO, which two of the following dyes would work best together? (For this question, assume that all dyes are excited with equal efficiency and emit at the same intensity.)

   (Circle only two)

a. Dye A	**(ex 490, em 520)**
b. Dye B	(ex 546, em 572)
c. Dye C	(ex 580, em 605)
d. Dye D	**(ex 650, em 667)**
e. Dye E	(ex 578, em 602)

3. The FMBIO allows the user to change the sensitivity of the photo multiplier tubes, depending on the dye being detected. Based on the 532 nm excitation source, which of the following dyes would most likely require a maximum (100%) detection sensitivity setting?

   (Circle only two)

a. Dye A	**(ex 490, em 520)**
b. Dye B	(ex 546, em 572)
c. Dye C	(ex 580, em 605)
d. Dye D	**(ex 650, em 667)**
e. Dye E	(ex 578, em 602)

4. Assuming a one-color detection system, choose the best filter for each dye. (You may use each answer more than once.)

f Dye A	(ex 490, em 520)	a. 667 nm
b Dye B	(ex 546, em 572)	b. 585 nm
c Dye C	(ex 580, em 605)	c. 605 nm
a Dye D	(ex 650, em 667)	d. 625 nm
c Dye E	(ex 578, em 602)	e. 650 nm
		f. 505 nm

Image Analysis

Briefly define or explain the significance of the following:

5. Excitation wavelength

   Optimum wavelength at which a fluorophor is excited

6. Emission wavelength

   Wavelength of light given off by a fluorophor

7. 65,536
The maximum pixel value (2 to the 16th)

Complete each of the following sentences by circling the correct word from each set of choices:

8. If horizontal resolution is changed from 300 dpi to 150 dpi:

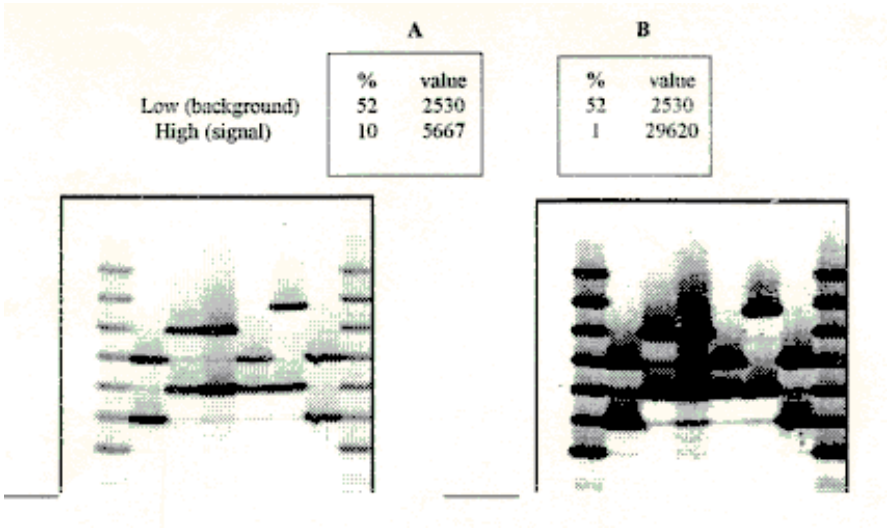
the image becomes: sharper / **more pixilated**

the image file size: **decreases** / increases

the scan time: **decreases** / increases

9. For best results, when reading a typing gel with closely-spaced bands, horizontal / **vertical** resolution should be set at 75 / 150 / **300** dpi.

10. Match the image to the Gray Scale settings.



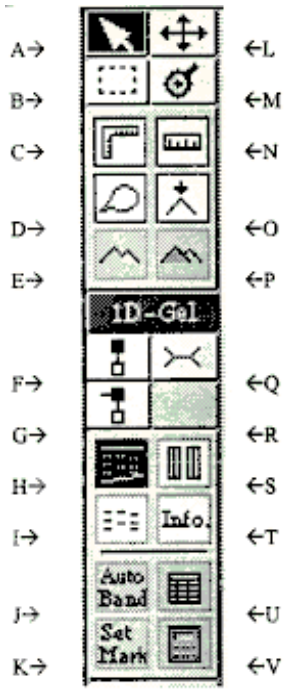
Explain what the "52%" Low (background) means.

52% of the pixels will be shown in white (all pixels with values < 2530 will be white).

Explain the difference between the 10% High (signal) and the 1% High (signal) as it relates to the gel scans above.

10% of the pixels with values > 5667 will be black.
1% of the pixels with values > 29620 will be black.

11. The following diagram represents commonly used icons on the FMBIO software. Match the function to the appropriate icon.



T a. Shows selected information next to bands on the gel file

M b. Magnifies the gel file picture

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J d. Defines bands within lanes automatically

D e. Comment icon, displays elements created in Fig Draw

E f. "Spectrum" tool, shows lane trace next to band image on the gel

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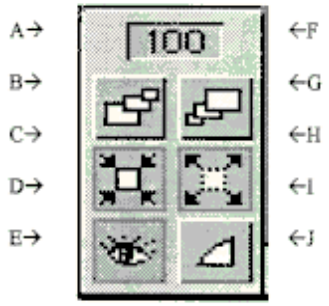

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13. If using an in-lane size marker labeled with a dye different from that used to label sample bands, the Separate / Mix / **Layer** Marker Mode should be set.

14. When using allelic ladders for band sizing, Marker Mode can be set to:

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d. a or b
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15. Your colleague needs your help. During an analysis, very few of the bands that are visible to his eye were called using the AutoBand button. Which of the following might help him identify more bands?

Mark true or false, and explain why or why not.

_T_ a. Choose AutoBanding Parameters and decrease the noise level.
Explain: Will allow more bands (peaks) to be identified

_F_ b. Choose AutoBanding Parameters and increase the noise level.
Explain: Will most likely miss lower peaks

_T_ c. Readjust any lane indicator lines that do not go through the middle of the lane.
Explain: Lane indicator may not be lined up with all bands and peaks may be missed

T d. Rescan the gel with the Sensitivity increased.
Explain: Increasing sensitivity increases signal value so bands can be found

T e. Manually input bands with the >-< tool.
Explain: Allows bands to be identified for analysis

16. Although the greater majority of the allele calls in your colleague's analysis of his data in STaRCall™ has resulted in accurate allele calls, you notice there are several "not in range" messages for some bands. Which of the following could be causing this? (Check all that apply.)

√ The incorrect lookup table was used for the data being analyzed.

√ The internal size markers were incorrectly identified (325 bp band was identified as 350 bp, etc.).

√ In FMBIO Analysis, some bands in the allelic ladder lanes were incorrectly identified.

√ In the lookup table, +/- values were too small.

√ Overloaded lanes resulted in inaccurate migration distances.

√ Manually banding lanes without the peak finder tool resulted in bands not positioned correctly.

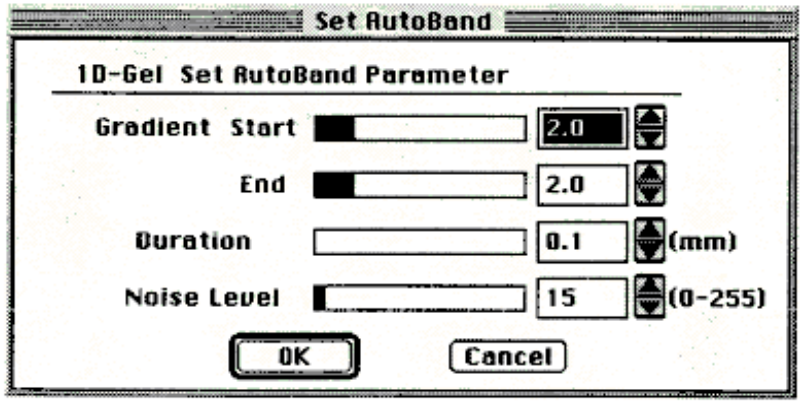
____ The gel was never color separated.

√ He has discovered new microvariants which are not present in the lookup table.

√ The CTTv lookup table was used to evaluate a lane that contained CSF, TPOX, amelogenin, TH01 and vWA.

√ Allelic ladders (instead of internal size markers) were used to size unknowns in a "smiling" or "frowning" gel.

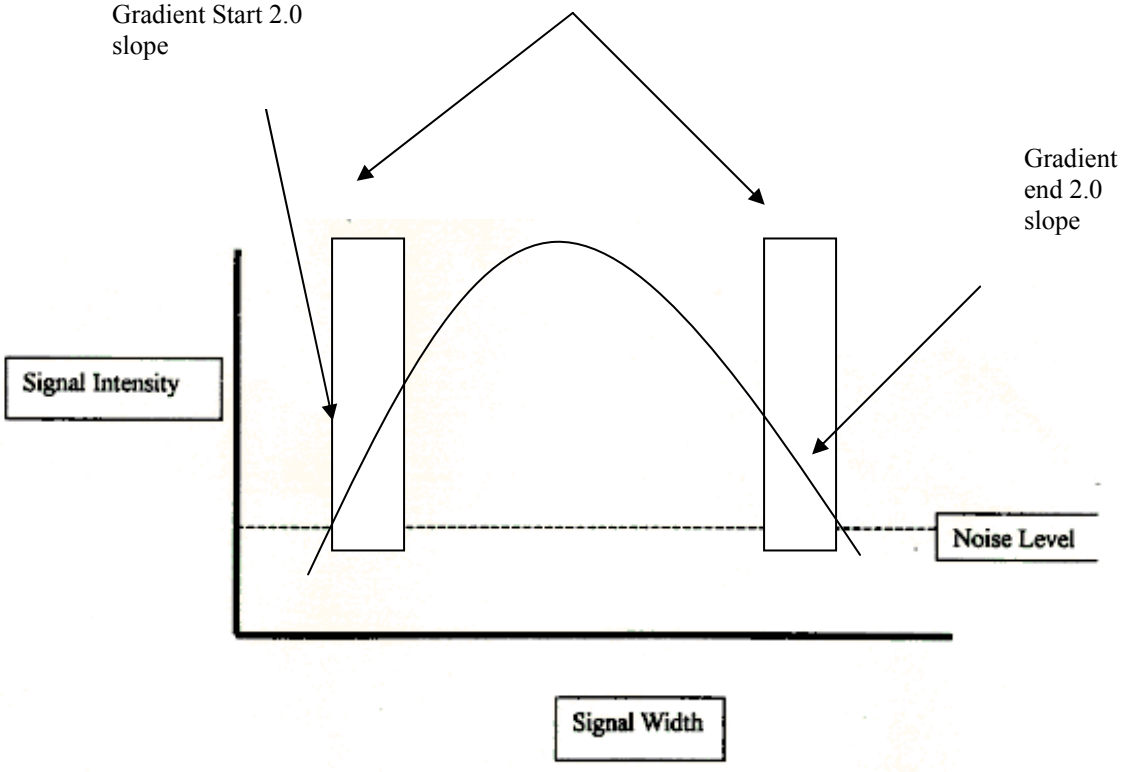
17. Band recognition depends on four parameters: Gradient Start, Gradient End, Duration and Noise Level. FMBIO Analysis recognizes a signal peak as a band by using a spectrum curve. Based on the information given in the diagram below, fill in the spectrum curve below that represents the settings shown in the "Set AutoBand" window.



Duration 0.1

Gradient Start 2.0 slope

Gradient end 2.0 slope



♦END